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Patent
Attorney's Docket No. 017753-152

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of	
Christian DEVAUX et al.	Group Art Unit: 1648
Application No.: 09/648,557	Examiner: Jeffrey S. Parkin
Filed: August 25, 2000	Confirmation No.: 5736
For: INHIBITORS OF HIV REPLICATION AND METHOD OF TREATMENT OF HIV INFECTIONS	

DECLARATION BY GILLES DIVITA PURSUANT TO 37 C.F.R. § 1.132

Assistant Commissioner for Patents
Washington, D.C. 20231

Sir:

I, Gilles Divita, Ph.D., declare as follows:

1. I am currently a research director at the Centre de Recherche en Biochimie Macromoléculaire, Biophysics Department.

2. I am an expert in the field of regulation of HIV infection by targeting reverse transcriptase activation, as substantiated by my curriculum vitae (attached hereto).

3. I am an inventor of and am familiar with the above-identified application.

4. In my opinion, Table 1 of our application readily demonstrates the high degree of conservation in the amino acids corresponding to the p7 region of reverse transcriptase of various strains of Human Immunodeficiency Virus (HIV). Table 1 of the application shows conservation of the tryptophan residues and of the basic (positively charged) amino acid in position 1. The data in the application relating to peptide p7 (a.k.a.

P-7) indicate that this peptide inhibits the replication of HIV, especially when complexed to MPG to enable entry into cells.

5. In my opinion, from the disclosure of the application, and in particular, Table 1, it would be clear to one skilled in the art that the main amino acids in this peptide responsible for the RT-inhibition effect are those that are clearly conserved between different strains of HIV.

6. Based on my qualifications and my familiarity with our application, I am qualified to attest to the accuracy and interpretation of additional tests that have been conducted confirming the motif present in the reverse-transcriptase inhibiting peptides exemplified in the application.

7. To confirm the conservation of sequence demonstrated in the application, studies were performed on a series of peptides corresponding to various mutations in the sequence of the P-7 peptide. These experiments were performed on MAGIC-5 cells according to the procedure described in Example 1.5 of the application. The HeLa-LTR-β-Gal indicator cell line stably transfected with CD4 and CCR5 (MAGIC-5 cells) previously described in Rey et al., *Biochem. Biophys. Res. Comm.* 121:126-133 (1984) was grown in DMEM containing 1% PS, 1% glutamax, 1 mg/ml G418, and 10% FCS. MAGIC-5 cells expressing the β-gal reporter gene cloned downstream of the HIV-1 LTR promoter were plated in 24-well plates at 5 x 10^5 cells/ml and incubated with 50 μl of stock HIV preparation corresponding to 1000 x 50% tissue culture infective dose ($TCID_{50}$)/ml in the presence or absence of MPG/Peptide (at 10^{-9} and 10^{-8}M). After 3 days in culture, cells were lysed and β-gal activity was determined by incubating 200 μl of total cellular extracts

for 1 hour at 37°C in 1.5 ml buffer containing 80 mM Na_2HPO_4, 10 mM $MgCl_2$, 1 mM 2 β-mercapto-ethanol and 6 mM O-nitrophenyl-β-D-galactopyranoside (ONPG). β-gal activity was evaluated by measuring absorbance at 410 nm. The effect of peptides bearing various mutations in the P-7 sequence on Reverse Transcriptase (RT) activity is shown in Table A.

Table A. Mutations in the P-7 Sequence Corresponding to RT Inhibition

Peptide	Sequence	Inhibition (%)
P-7	KETWETWWTE	98.7
P-71	KETAETWWTE	99.6
P-72	KETWETAWTE	99.6
P-73	KETWETWATE	99.7
✓ P-74	KETWETAATE	14.8
P-75	KETWATWWTE	99.3
✓ P-76	KETAETWATE	12.7
✓ P-77	KETAETAATE	0
P-78	KATWETWWTE	99.5
P-79	KEAWETWWTE	99.5
✓ P-80	KETAETAWTE	0
P-81	KETWETWWT	99
P-82	RETWETWWTE	95

8. Table A shows the essential role of tryptophans in the inhibitive character of P-7. The alteration of two out of three tryptophans leads to a loss of inhibition (peptides P-74, P-76, P-77, and P-80).

9. Table A also demonstrates that modification of amino acids in position 2, 3, 5, and 6 do not affect the inhibitory power of P-7 (P-75, P-78, P-79). On the other hand, a positive (basic) residue (lysine or arginine) in position 1 is indispensable for P-7 activity. The alteration of this residue to alanine reduces dramatically the inhibitory power of P-7. However the alteration of the original lysine to arginine (R) results in retention of 95% inhibition.

10. Finally, Table A demonstrates that the glutamic acid residue in position 10 is not essential for the inhibitory effect (P-81).

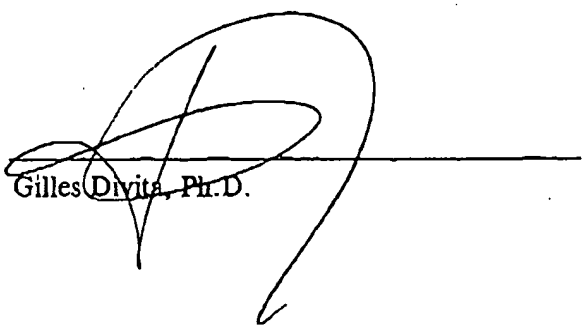
11. These inhibition results confirm the importance of the role of tryptophans, and the low level of importance of modifications of positions 2, 3, 5, 6, and 10. The low level of importance of a modification of position 9 was previously demonstrated in Table 1 of the instant specification, as there is a naturally high variability of amino acids in this position.

12. The above-indicated data thus confirm the assertion in the application (e.g., page 4, Description of the Preferred Embodiments) that only certain residues are important in the decapeptides of the present invention. These residues are clearly identified in the application and in the claims. The broadest claim (Claim 1) encompasses a decapeptide with, from the N-terminus to the C-terminus, a basic amino acid in position 1, an acidic amino acid in positions 2 and 5, and a tryptophan in positions 4, 7, and 8. I and the other inventors have clearly identified these residues in the decapeptide in the application as the important residues and have shown that changes in the other positions do not impact upon

the ability of the decapeptide to inhibit reverse transcriptase. Thus, the presently claimed invention is adequately described by the application and claims.

13. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

23/01/03
Date



Gilles Divita, Ph.D.